Local proliferation of monocytes

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Resident macrophages are found in essentially all tissues and play key roles in tissue development and homeostasis as well as during inflammation and regeneration. Dependent on the tissue, adult tissue-resident macrophages may arise from embryonic hematopoiesis and subsequently are maintained in the tissues by local self-renewal (1). Alternatively, tissue-resident macrophages may arise from adult bone marrow-derived monocytes, which locally differentiate into macrophages (1). For example, while brain-resident microglia remain of embryonic origin over the life-course, a substantial fraction of gut and skin resident macrophages are regularly replaced by bone marrow-derived monocytes (1).

Consistent with an ability to self-maintain, macrophages also exhibit a profound capacity to proliferate under inflammatory conditions, as shown for example in the serous cavities (2). This is not a feature restricted to either cells of embryonic or bone marrow origin (3) but a process dictated by the local microenvironment. Under inflammatory conditions, such as following wounding, an influx of leukocyte populations from the blood occurs. These cells play different roles dependent on the stage of wound repair, and include monocytes that differentiate and lead to a transient expansion of local macrophage populations. Monocyte-derived cells recruited during inflammation can also proliferate extensively (4). Hence, dependent on inflammatory context, local macrophage numbers may increase due to differentiation of recruited monocytes or due to the proliferation of tissue-resident and recruited inflammatory macrophage populations (4).

During cutaneous wound healing, the physiological relevance of macrophages is well-established (5). However, the relative contribution of recruitment and/or proliferation of macrophage numbers during cutaneous wound repair has remained unclear. Furthermore, while monocytes within the bone marrow undergo extensive proliferation those within the blood do not (6). This has led to the general conception that outside of the bone marrow, proliferation may only be the preserve of differentiated macrophages. Countering this view, Pang *et al.* now show that Ly6C-expressing myeloid cells recruited to the site of an excisional skin wound undergo extensive proliferation (7). These findings complement two recent studies that found urinary tract (8) and hepatic (9) monocytes to proliferate during bacterial and helminth infection respectively, suggesting bone marrow-derived monocytes are able to proliferate to a significant degree within certain inflamed tissues. Critically, Pang *et al*. show that immigrating Ly6C+ cells proliferate more than the fully differentiated F4/80-expressing tissue-resident macrophages; suggesting that monocyte proliferation prior to differentiation may substantially contribute to the expansion of macrophages during inflammation (Figure 1).

These findings substantially add to our understanding of the dynamics of monocyte recruitment and macrophage generation during inflammation and change our view of how monocytes may function during wound healing. These findings also raise a wide range of new questions.

Most prominently, it would be important to better understand the characteristic of this proliferating monocyte population. Currently, we assume that these proliferating Ly6C$^+$ cells contribute to an expansion of the tissue monocytes and the macrophage populations they seed; however, their fate following proliferation remains unknown. Specifically, it remains to be shown whether these proliferating cells indeed differentiate into macrophages and to what extent this proliferation contributes to the further generation of monocytes and macrophages. In this respect, a formal demonstration of whether monocyte proliferation is actually essential for the expansion of macrophage populations during cutaneous injury appears warranted. Notably, in a model of pancreatic injury, prevention of monocyte influx resulted in exaggerated proliferation of the remaining tissue-resident macrophages, which compensated for the lack of monocyte differentiation (10). Hence, local monocyte proliferation may similarly be redundant, and it would be interesting to see whether enhanced monocyte recruitment or proliferation of differentiated macrophages occurred, if monocyte proliferation at the site of inflammation were blocked.

Furthermore, one of the most conceptually interesting questions raised by the apparent proliferation of tissue monocytes is why these cells do not proliferate in the blood. In agreement with previous studies (6), Pang *et al*. demonstrate that Ly6C+ monocytes proliferate in the bone marrow but not the blood. One possibility could be that the tissue and bone marrow environments provide signals that drive proliferation (e.g. mitogens in appropriate quantities); alternatively, monocytes within the blood could actively be inhibited from proliferating. In this regard, CSF1 is a well characterized macrophage mitogen and Ly6C+ blood monocytes are able to outcompete Ly6C- monocytes for capture of circulating CSF1, yet they remain non-proliferative. However, it also remains unclear whether those Ly6C+ cells that proliferate within the healing skin should actually be considered monocytes. This assumption is based solely on their retention of the marker Ly6C. The cells in this study were isolated at day 3 post-injury and hence it is possible they may have already undergone a significant degree of differentiation. Notably, Schridde *et al.* found that Ly6C+ cells isolated from healthy skin and gut are transcriptionally distinct from those in the blood and exhibit tissue-specific transcriptional signatures (11). Hence, it will be important to determine whether monocytes must first undergo as yet undefined steps of differentiation within the tissues in order to be able to re-enter cell cycle. Addressing these points will be important for understanding the molecular machinery involved in triggering proliferation of the Ly6C$^+$ population within the tissue.

Similarly, it will be valuable to identify those tissue factors that positively regulate monocyte/macrophage proliferation within the healing dermis. Pang *et al*. demonstrate that proliferation is not affected in IL1 or IL6-deficient mice, suggesting these factors are non-essential or redundant in driving monocyte proliferation. Pang et al also make the case that IL-4 and CSF1 are unlikely to be involved, based on evidence that CD115 is not detected on skin monocytes/macrophages obtained from digested wounds and

that tissue levels of IL-4 or CSF1 are unchanged or even decrease within the skin following injury. However, formal demonstration that proliferation of monocytes is independent of CSF1 or IL-4 is lacking. Unfortunately, it is well known that surface CD115 (*Csf1r*) is difficult to detect by flow cytometry on macrophages isolated from tissues by enzymatic digestion, despite expression of CSF1R by these cells *in vivo*. Furthermore, a detected loss of measurable CSF1 in tissues could reflect an elevated consumption of CSF1 that would be predicted to occur with influx of monocytes. Notably, *in vivo* blockade of CSF1 consumption by treatment with anti-CSF1R mAb has revealed increased production of CSF1 following inflammation that had otherwise remained undetectable (2). Similarly, IL-4R ligands is notoriously difficult to detect *in vivo* despite measurable effects on cells including macrophages. Indeed, myeloid cell-expression of IL-4 receptor has already been demonstrated to be important in the proliferation and accumulation of F4/80+ macrophages within excisional skin wounds (12), and hence a role for this cytokine in the proliferation of $Ly6C^{+}$ cells seems highly likely.

One final intriguing question posed by this study is why Ly6C+ monocytes/macrophages proliferate more than F4/80+ macrophages within the skin wounds. The authors propose that monocytes may be inherently more proliferative than mature Ly6C- cells. This would be an important conceptual advance and in line with published data that showed that resident peritoneal cavity macrophages of recent monocyte-origin exhibit higher levels of proliferation than incumbent resident population (3). However, as previously mentioned, the potential to proliferate is known to be determined by the immediate microenvironment within which macrophages reside. Hence, it will be important to determine whether Ly6C+ and F4/80+ dermal cells differ in their location within the injured tissue and with which cells they interact within their microenvironment. Thus, it will be critical to determine whether the proliferating cells are in close physical proximity to cytokine producing cell types or whether they may have recently been exposed to mitogens, such as IL-4.

Taken together, the findings by Pang *et al.* open a new perspective on the process of wound healing and raise a wide range of novel questions, which will be exciting to address in future research.

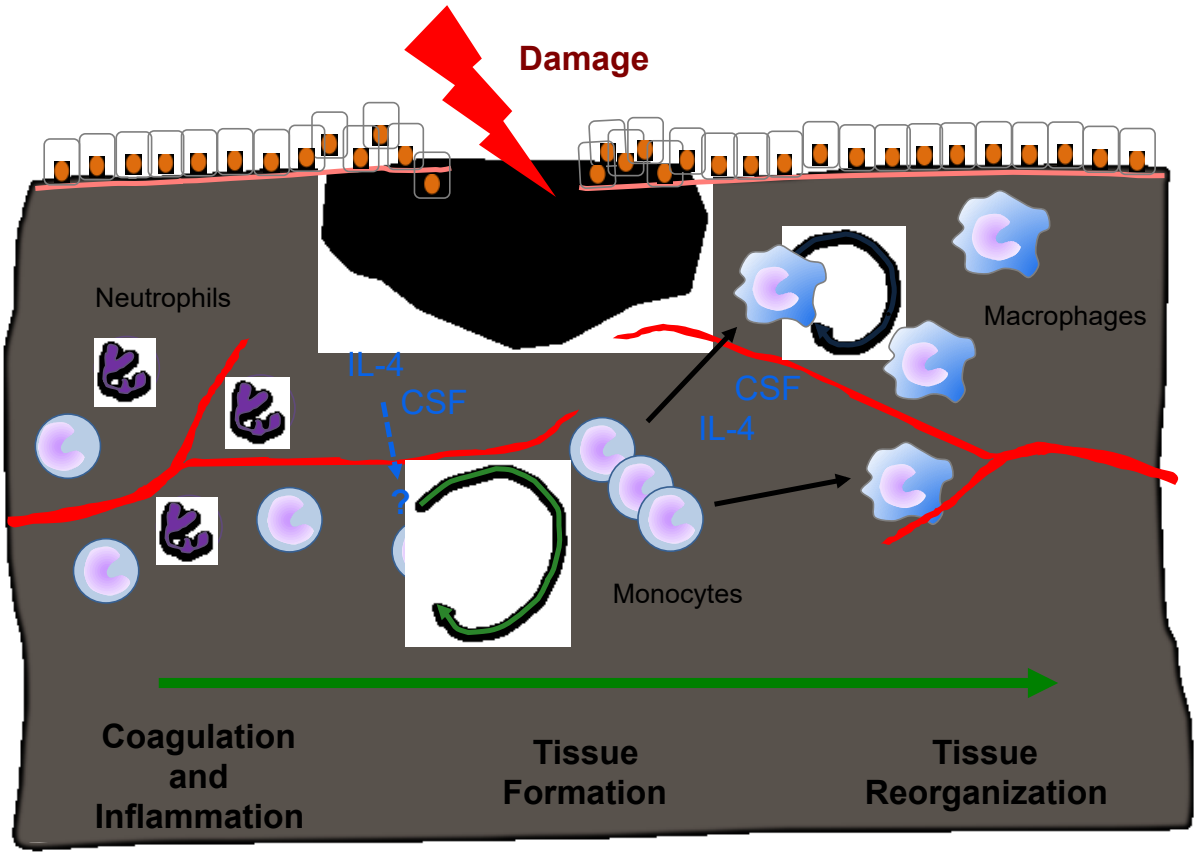

Figure 1: Monocyte proliferation contributes to local macrophage expansion. Following skin wounding a highly dynamic, but also tightly coordinated process of tissue restoration unfolds. This process can be divided into three overlapping but distinct phases. During the “Coagulation and Inflammation Phase” inflammatory cells, such as neutrophils or monocytes, are recruited to the damaged tissue. Once monocytes have infiltrated the tissue, an IL-4 mediated monocyte differentiation into macrophages is initiated during “Tissue Formation Phase”, substantially contributing to the CSF-mediated expansion of the macrophage population within the wounded tissue, which then critically contributes to the resolution of local inflammation and to tissue restructuring during the final “Tissue Remodeling Phase”. So far, it had been assumed that monocytes mainly proliferate in the bone marrow and then, once fully differentiated, immigrate the site of tissue damage. Recent publications however now suggest that monocytes can also proliferate during inflammation, thereby contributing to the expansion of the monocyte population at the site of tissue damage.

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